APPLICATION INVOLVING RHIZOBIUM AND AZOTOBACTOR

Week 1 (2015/06/21-2015/06/27)

1. Bacteria transformation for phosphate sensor testing

Week 2 (2015/07/19-2015/07/25)

1. Made media for rhizobium and azotobacter Tx pSH033 Cl into *E. coli* XL1 EC

2. Made 1 L TY Media

3. Made 500 mL YMB Media

4. Made 500 mL of Azotobacter Media

5. Made 16 plates of Azotobacter Media

6. Made 16 plates of YMA

7. Transformed pSH033 Cl plasmid into E. coli XL1 EC.

8. Ramya plated 100 microliters and 100 microliters of 1:100 dilution on LB/Chlor(17) plates. Incubated at 37 C

9. Grew glycerol stock of Rhizobium on toothpick in 3 mL YMB at RT and 250 rpm for 3 days.

10. Transformed pSH033 into S17 E. coli Heat Shock Competent Cells by heat shock protocol. Incubated 1 h at 250 rpm and 37C in 1 mL SOC and plated 100 microliters and the rest on LB/Chl(17) plates.

11. 1 Colony on LB/Chl(17) plate with 900 microliters.

Week 3 (2015/07/26-2015/08/01)

1. Grew cultures of pSH033 in XL1 EC (2), pSH033 + pET28a in SH17 (2), and pSH033 in SH17 (from the plate transformed on July 24th) in 3 mL LB/Chl(17).

2. Streaked rhizobia on plate and put in incubator at 27 C for 3 days.

3. Cultures of pSH033 in XL1 EC (2) grew, but cultures of pSH033 + pET28a in SH17 (2), and pSH033 in SH17 (from the plate transformed on July 24th) did not grow.

4. Miniprepped pSH033 in XL1 EC and measured concentrations by nanodrop. (QIAGEN Miniprep Kit)

5. Made glycerol stocks (500 microliters 50% glycerol and 500 microliters culture) of pSH033 in XL1 EC and rhizobia. Put in -80C box

6. Streaked LB plate with frozen SH17 (glycerol stock) to make heat shock competent cells and Incubated 22 h at 37 C.

7. Made heat shock competent S17 E.coli cells

8. Grew 3 mL LB/Chl(34) pSH033 in S17. Grew 3 mL LB/Tet(10) pAM120 in S17 from 9:15 A.M. at 37C, 250 rpm.

9. What I need to do next time: Conjugation of rhizobia and E. coli cells

10. Transcribing and translating mCherry without cells